

UNCLASSIFIED

AD NUMBER
ADB275101
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; Oct 2001. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott St., Ft. Detrick, MD 21702-5012.
AUTHORITY
USAMRMC ltr, 28 Aug 2002

THIS PAGE IS UNCLASSIFIED

AD_____

Award Number: DAMD17-00-1-0634

TITLE: Targeting ID to Inhibit the Vascularization of Breast Tumors

PRINCIPAL INVESTIGATOR: Robert Benezra, Ph.D.

CONTRACTING ORGANIZATION: Sloan-Kettering Institute for Cancer Research
New York, New York 10021

REPORT DATE: October 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Oct 01). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20020215 031

NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-00-1-0634

Organization: Sloan-Kettering Institute for Cancer Research

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Kath Mome 1/18/02

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 2001	3. REPORT TYPE AND DATES COVERED Final (25 Sep 00 - 24 Sep 01)	
4. TITLE AND SUBTITLE Targeting ID to Inhibit the Vascularization of Breast Tumors			5. FUNDING NUMBERS DAMD17-00-1-0634	
6. AUTHOR(S) Robert Benezra, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Sloan-Kettering Institute for Cancer Research New York, New York 10021 E-Mail: r-benezra@ski.mskcc.org			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Distribution authorized to U.S. Government agencies only (proprietary information, Oct 01). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Under the auspices of this Army Concept Award we have begun the derivatization of a compound (AIL, for anti-Id lead) shown to bind to the Id proteins, known to be required for the formation of blood vessels into breast tumors. We have identified sites on AIL that can be modified and have begun the chemistry of adding a linker to this region of the molecule that can accept geldanamycin. The AIL-geldanamycin fusion will be used to target the Id proteins for degradation in human cancers and thereby inhibit tumor angiogenesis.				
14. SUBJECT TERMS Id, Angiogenesis, Small molecule inhibitor				15. NUMBER OF PAGES 6
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	
SF 298.....	
Table of Contents.....	1
Introduction.....	2
Body.....	2-3
Key Research Accomplishments.....	3
Reportable Outcomes.....	3
Conclusions.....	3
References.....	N/A
Appendices.....	N/A

Introduction:

The Id proteins have recently been identified by my laboratory as being essential for the growth and maturation of blood vessels in murine breast tumor xenografts (1). Even partial loss of Id function leads to a profound reduction in the vascularization of tumors with a concomitant loss of growth and metastatic potential. These observations, the fact that Id proteins are expressed at very low levels in adult mice and humans and the fact that the precise mechanism of Id action has been defined make these proteins extremely attractive targets for anti-angiogenic drug design in the treatment of human cancers. The research being proposed here focuses on the novel derivatization of a lead compound which we have shown interacts with Id and inhibits its activity in vitro and in cell culture models. (called AIL for anti-Id lead) . The derivative compounds will be tested for their efficacy in the inhibition of the vasculature of breast cancer xenografts and genetically induced breast cancers in mice.

Thus, our recent observation that the Id proteins are required for the vascularization of breast cancer xenografts directs our search for novel compounds that can inhibit Id and thereby the growth and metastasis of breast tumors. The detailed understanding we have of the mechanism of action of Id will facilitate the identification of compounds that exhibit high specificity and low toxicity and therefore are likely to be important clinical tools in the treatment of this disease.

Body:

In order to derivatize the AIL-lead compound with geldanamycin, we first needed to determine which sites on the AIL could be modified without affecting its ability to interact with Id proteins. Five AIL-derivatives with different sites modified were tested for their ability to compete with ^3H -AIL as a measure of relative affinity. As shown in figure 1, several derivatives competed for ^3H -AIL as well as unlabelled native AIL whereas others did not. Based on the chemical structure of these derivatives we have now begun to focus our attention on the fusion of geldanamycin to linkers attached to sites on AIL that do not affect its activity. Thus far the linkers fused to AIL have proven to be yield chemically unstable derivatives (by spectroscopic measurements) but modification of those linkers is currently underway.

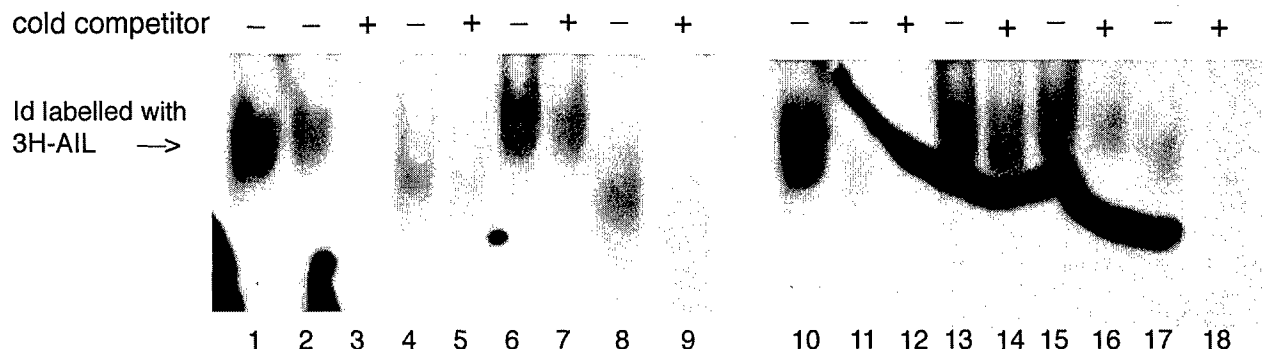


Figure 1. Competition assay to determine the ability of cold AIL derivatives to inhibit the binding of 3H-AIL to Id1. 3H-AIL was incubated with purified Id protein and the mixture analyzed by native gel electrophoresis. 3H-AIL bound to Id1 is visualized by autoradiography in the position indicated with the arrow. 3H-AIL alone does not enter the gel (not shown). Unlabelled derivatives of AIL are tested for their ability to inhibit the binding of 3H-AIL to Id1. Lanes 1,10: 3H-AIL with no competitor. Lanes 2,3: carrier for derivative 1 and 100 fold excess derivative 1, respectively. Lanes 4,5 and 6,7 and 8,9 and 17,18: carrier (lanes 4,6,8) and derivatives 2,3,4 and 5 (lanes 5,7,9 and 17,18). Lanes 11,12 and 13,14 and 15,16: carrier (lanes 11,13,15) plus 3 concentrations of cold AIL (lanes 12>14>16).

Further efforts should continue to produce more stable AIL-linker derivatives that will allow the fusion of AIL to geldanamycin and testing for its ability to inhibit Id activity.

Key Research Accomplishments:

- Development of a competition assay for the binding of AIL-derivatives to Id using 3H-AIL
- Identification of sites on the AIL suitable for linker attachment and derivatization

Reportable Outcomes:

This work has led to a more thorough development of an anti-Id strategy for the treatment of breast cancer. The ideas and results generated have helped me to secure a \$225,000 award from the Breast Cancer Research Foundation to continue this work.

Conclusions:

The development of an assay for the ability of AIL derivative to bind Id is an important step forward in the identification of compounds that can inhibit Id activity. This assay has facilitated the identification of sites on the AIL that will not perturb its ability to bind Id and should allow us to place linkers on the AIL for the purpose of fusion to geldanamycin. The linkers tested thus far have not produced stable compounds. Other linkers must now be tested and once stable compounds generated, fusion to geldanamycin can be performed and tested for maintenance of their ability to bind and inhibit Id. This may lead to compounds capable of inhibiting blood vessel formation around breast cancers.



DEPARTMENT OF THE ARMY

US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

28 Aug 02

MEMORANDUM FOR Administrator, Defense Technical Information
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
VA 22060-6218


SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl


PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

ADB231838
ADB240253
ADB251610
ADB275099
ADB253637
ADB261538
ADB275186
ADB264648
ADB275102
ADB241899
ADB259033
ADB266113
ADB275663
ADB254489
ADB262700
ADB276708
ADB274345
ADB274844
ADB275154
ADB275535
ADB275101
ADB275451
ADB274597
ADB273871
ADB275145
ADB274505
ADB275851
ADB274459
ADB277942
ADB277404
ADB277494
ADB277536